Supporting Information – Synthesis of Substrates

Arylsulfatase K is the Lysosomal Glucuronate-2-sulfate Sulfatase

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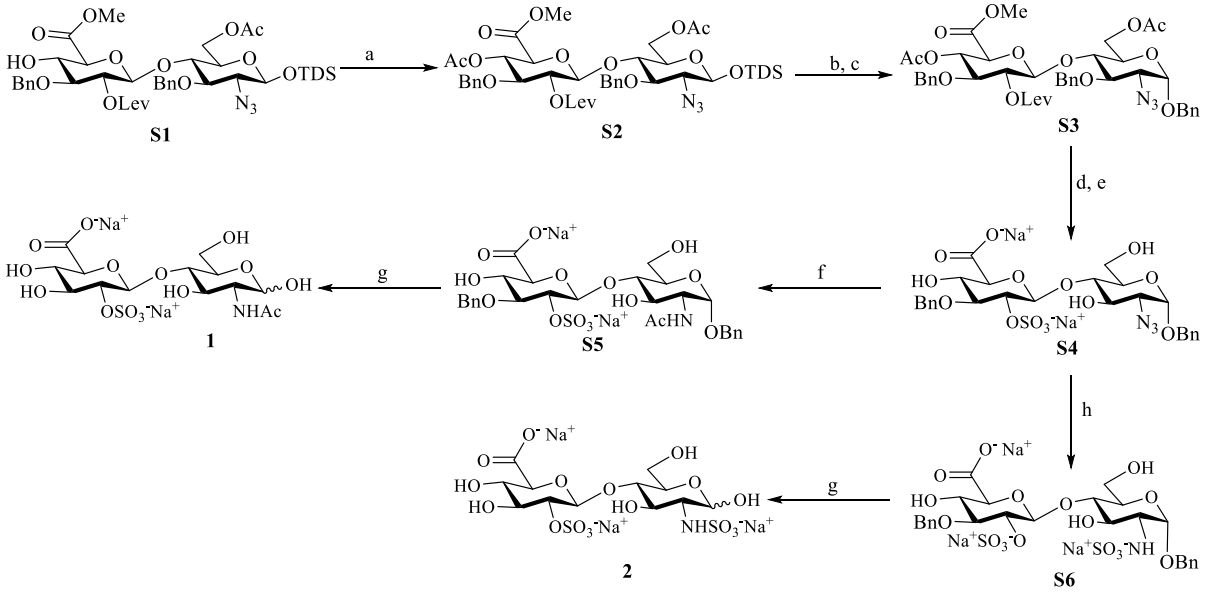
General procedures: All moisture sensitive reactions were performed under an atmosphere of argon and vacuum dried glassware. All commercial reagents were used without purification, unless otherwise noted. CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Toluene, DMF, diethyl ether, methanol and THF were purchased anhydrous and used without further purification. Molecular sieves (4Å) were flame activated *in vacuo* prior to use. All reactions were performed at room temperature unless specified otherwise. TLC analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV-absorption (254 nm) where applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 ^oC or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150^oC. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 µm, 60 Å) or on Bondapak C-18 (Waters). ^1H and ^{13}C NMR spectra were recorded on a Varian inova-300 (300/75 MHz), a Varian inova-500 (500/125 MHz) and a Varian inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basis of ^{1}HNMR, ^{13}C NMR, COSY and HSQC experiments. Mass spectra were recorded on an Applied Biosystems 5800 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxy-benzoicacid (DHB) and ultamark 1621 as the internal standard.



Scheme S1 - Synthesis of Compound 1 and 2. a) Pyridine, Ac_2O, 84%; b) HF. Py, 18 hr, 98%; c) (i) DBU, CCl_3CN, DCM, 70%; (ii) BnOH, TMSOTf, Et_2O, 100%; d) (i) NH_2NH_2.HOAc, Toluene/Ethanol; (ii) Py·SO_3, DMF; e) H_2O_2, LiOH 0.22 M, THF, 50% for three steps; f) PMe_3, NaOH, THF; (ii) Et_3N, Ac_2O, methanol, 73%; f) $Pd(OH)_2$/C, H_2, (38%, **1**; 100%, **2**); g) PMe_3, NaOH, THF; (ii) Et_3N, SO_3Py, NaOH, 78%.

Dimethylthexylsilyl *O*-(methyl-4-*O*-acetyl-2-*O*-levulinoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-acetyl-β-D-glucopyranoside (S2): A solution of **S1** [1] (360 mg, 0.419 mmol) in pyridine and acetic anhydride (4/1, v/v, 0.2 M) was stirred for 6 h at ambient temperature. TLC (toluene/EtOAC, 60/40, v,v) indicated the consumption of the starting material, after which the mixture was concentrated in *vacuo*. The residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc (70/30, v/v) to obtain **S2** (318 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 6.97 (m, 10H, CH Aromatic), 5.00 (t, *J* = 9.5 Hz, 1H, H2'), 4.89 (t, *J* = 9.0 Hz, 1H, H4'), 4.84 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.62 (d, *J* = 11.6 Hz, 1H, CH*H*Ph), 4.53 – 4.39 (m, 4H, H1, CH_2Ph, H5'), 4.33 (d, *J* = 7.7 Hz, 1H, H1'), 4.25 (d, *J* = 11.7 Hz, 2H, H6), 4.06 – 3.90 (m, 1H, H5), 3.58 – 3.00 (m, 1H, H2, H3, H4, H3', COCH_3), 2.63 – 2.16 (m, 4H, CH_2 of Lev), 1.99 (s, 3H, COCH_3), 1.91 (s, 3H, COCH_3), 1.76 (s, 3H, COCH_3), 1.50 – 2.34 (m, 1H, C*H* of TDS), 0.90 – 0.70 (m, 12H, CH_3 of TDS), 0.01 (d, *J* = 6.7 Hz, 6H, CH_3 of TDS). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.26, 171.41, 170.83, 169.56, 167.38, 138.90, 137.96, 128.61, 128.44, 128.01, 127.89, 127.52, 127.49, 101.23, 96.94, 81.23, 79.88, 78.97, 77.71, 77.29, 76.86, 75.11, 74.43, 73.15, 73.12, 72.65, 71.09, 68.88, 62.92, 52.86, 37.79, 34.15, 30.01, 27.88, 25.02, 21.05, 20.80, 20.17, 20.08, 18.69, 18.59, -2.00, -3.07. HRMS MALDI-TOF: ($M+Na^+$) found 922.3770, observed 922.3792.

Benzyl *O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-acetyl-α-D-glucopyranoside (S3): HF·Py complex was added to a solution of compound **18** (0.48 g, 0.61 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature for 18 h when TLC (toluene/EtOAC, 60/40, v/v) indicated consumption of the starting material. The reaction was quenched with water, diluted with DCM and extracted with sodium bicarbonate and washed with water for 3 times. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in *vacuo*. The residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc (70/30, v/v) to obtain *O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl-4-*O*-(9-*O*-acetyl)-β-D-glucopyranosyluronate)-(1→4)-*O*-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-acetyl-α/β-D-glucopyranoside (385 mg, 98%). Trichloroacetonitrile (5 mL) and DBU (17.03 mg, 0.112 mmol) were added at to a cooled (°0 C) solution of the lactol (240 mg, 0.373 mmol) in DCM (5 mL) and stirred for 2 h until TLC (toluene/EtOAC, 60/40, v, v) indicated the consumption of the starting material. The reaction mixture was concentrated in *vacuo* and the residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc (60/40, v/v) to give the trichloroacetimidate (263 mg, 70%). The trichloroacetimidate donor (51 mg, 0.064 mmol) and benzyl alcohol (10.38 mg, 0.096 mmol) were combined in a flask, co-evaporated with toluene (3 x 2 mL) and dissolved in ether (1 mL). Powdered freshly activated 4 A molecular sieves were added and the mixture was stirred for 30 min at ambient temperature and then cooled to -20 °C. TMSOTf (0.2 equiv) was added, and stirring was continued until TLC indicated the disappearance of donor (~15 min). The reaction was allowed to warm to 5^{o}C and then quenched by the addition of DTBMP. The mixture was filtered, the filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc (60/40, v/v) to give compound **S3** in quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 – 7.20 (m, 15H, C*H* Aromatic), 5.10 (t, *J* = 9.0 Hz, 1H, H2'), 4.94 – 4.69 (m, 2H, H4', C*H*HPh), 4.68 – 4.53 (m, 1H, CH*H*Ph), 4.50 – 4.40 (m, 6H, 2*CH_2Ph, H1', H5'), 4.30 (d, *J* = 8.6 Hz, 1H, CH_2Ph), 4.25 (d, *J* = 9.3 Hz, 1H, H1), 4.08 – 3.96 (m, 2H, H6), 3.84 – 3.00 (m, 8H, H2, H3, H4, H5, H3', COOCH_3), 2.76 – 2.00 (m, 4H, CH_2 of Lev), 2.12 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 1.93 (s, 3H, COCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.90, 137.96, 128.61, 128.44, 128.01, 127.89, 127.52, 127.49, 101.23, 96.94, 81.23, 79.88, 78.97, 77.71, 77.29, 76.86, 75.11, 74.43, 73.15, 73.12, 72.65, 71.09, 68.88, 62.92, 52.86, 37.79, 34.15, 30.01, 27.88, 25.02, 21.05, 20.80, 20.17, 20.08, 18.69, 18.59. HRMS MALDI-TOF: ($M+Na^+$) found 870.3061, observed 870.3071.

Benzyl *O*-(methyl-2-*O*-sulfonato-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (S4): Anhydrous hydrazine acetate was

added to a solution of compound **S3** (97.0 mg, 0.11 mmol) in a mixture of ethanol and toluene (2/1, v/v, 6 mL). Stirring was continued until TLC indicated the consumption of the starting material (~2 h). Acetone (0.5 mL), EtOAc (5 mL) and water (5 mL) were added and the organic layer was separated, dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. A mixture of SO_3·Py (167mg, 1.1 mmol) in 2 mL DMF was stirred at room temperature for 2 h, followed by the addition of pyridine and methanol. The mixture stirred for 20 minutes and concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was redissolved in a mixture of THF (2.0 mL) and 1.0 M NaOH (1 mL). The reaction mixture was left stirring at room temperature for 30 min. The pH was then adjusted to 9.0 by the addition of acetic acid followed by concentration *in vacuo* (bath temperature 20 ^oC) and the residue was applied to RP-18 silica gel column, which was eluted with a stepwise gradient of water and methanol (from 90/10 to 50/50, v/v). The appropriate fractions were concentrated *in vacuo* to give **S4** (37 mg, 50%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 – 7.17(m, 15H, CH Aromatic), 5.23 – 5.03 (m, 1H, H2', 3*CH_2Ph), 4.75 – 3.50 (m, 1H, H1, H2, H3, H4, H5, H6, H1', H3', H4', H5'). ^{13}C NMR (75 MHz, CD_3OD) δ 174.97, 139.09, 138.60, 137.35, 128.58, 128.53, 128.18, 128.08, 128.02, 127.72, 127.61, 127.35, 127.08, 101.22, 96.97, 83.36, 80.58, 78.17, 76.50, 75.10, 74.45, 74.39, 72.37, 71.97, 69.15, 63.03, 60.04. HRMS ESI-TOF: (M-Na^+) found 662.1273, observed 662.1285.

Benzyl *O*-(2-*O*-sulfonato-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (S5): A 1.0 M solution of PM_3 in THF and a 0.1 M NaOH solution was added to compound **S4** (18.5 mg, 0.024 mmol) in THF. The reaction mixture was stirred at room temperature for 1 h until TLC indicated completion of the reaction using ninhydrin as visualizing agent. The pH was adjusted to 9.0 by careful addition of acetic acid, and the resulting mixture was concentrated *in vacuo* (bath temperature 20 ^oC). Acetic anhydride (10 equiv. per NH_2) was added to a solution crude starting material in anhydrous methanol (0.8 mL) and triethyl amine (20 equiv. per NH_2) at 0 ^oC. The reaction was left stirring at room temperature for 1 h. The mixture was coevaporated with toluene and the residue was passed through a AG50W resin (Bio-Rad, 0.6 × 5 cm) using a mixture of CH_3OH and H_2O (90/10, v/v) as eluent, and appropriate fractions were concentrated in vacuo. The residue was vortexed with water and applied to a C-18 column, which was eluted with a stepwise gradient of H_2O and CH_3OH (from 90/10 to 40/60, v/v). The appropriate fractions were concentrated under reduced pressure to give **S5** (13.8 mg, 73%).^1H NMR (500 MHz, CD_3OD) δ 7.34 – 7.08 (m, 15H, CH Aromatic), 5.00 – 4.50 (m, 6H, H1, H1', H2', H5', 3*H of CH_2Ph), 4.39 (d, *J* = 12.0 Hz, 1H, CH*H*Ph), 4.27 (t, *J* = 8.4 Hz, 1H, H2), 4.06 – 3.00 (m, 7H, H3, H4, H5, H6, H3', H4'), 1.74 (s, 3H, COCH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 174.81, 172.00, 138.75, 138.08, 137.26, 128.38, 128.29, 128.08, 127.99, 127.51, 127.48, 127.44, 126.90, 100.83, 96.11, 83.04, 80.11, 79.03, 75.88, 74.78, 74.20, 74.09, 72.07, 71.81, 68.81, 59.72, 52.80, 21.30. HRMS ESI-TOF: (M-Na^+) found 678.1474, observed 678.1465.

Benzyl *O*-(2-*O*-sulfonato-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-*N*-sulfonato-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (S6): A 1.0 M solution of PM_3 in THF and a 0.1 M NaOH solution was added to compound **S4** (22 mg, 0.029) in THF. The reaction mixture was stirred at room temperature for 1 after until TLC indicated the completion of the reaction using ninhydrin as visualizing agent. The pH was adjusted to 9.0 by careful addition of 0.1 M HCl, and the mixture was concentrated *in vacuo* (bath temperature 20 ^oC). SO_3·Py (5 equiv per NH_2) was added to a solution of the starting material in methanol, triethyl amine and 0.1 M NaOH (2 equiv per NH_2) at 0 ^oC. The progress of the reaction was monitored by TLC .The reaction mixture was left stirring at room temperature for 2 h, after which it was co-evaporated with water. The residue was passed through a AG50W resin (Bio-Rad, 0.6 x 5 cm) using methanol and water (90/10, v/v) as the eluent. Appropriate fractions were concentrated *in vacuo* and then applied to a RP-18 column, which was eluted with a stepwise gradient of water and methanol (from 90/10 to 50/50, v/v). The appropriate fractions were concentrated *in vacuo* to give **S6** (9 mg, 78%). ^1H NMR (600 MHz, CD_3OD) δ 7.49 – 7.04 (m, 15H, CH Aromatic), 5.14 (d, *J* = 3.7 Hz, 1H, H1), 5.03 – 3.00 (m, 17H, H2, H3, H4,

H5, H6, H1', H2', H3', H4', H5', 3*CH_2Ph). ^{13}C NMR (151 MHz, CD_3OD) δ 174.77, 138.82, 137.65, 137.57, 129.07, 128.47, 128.15, 127.99, 127.83, 127.70, 127.55, 127.30, 126.94, 100.80, 98.04, 83.13, 80.18, 79.09, 75.89, 75.30, 74.32, 73.49, 72.10, 71.85, 69.81, 59.73, 57.98. HRMS ESI-TOF: (M-Na^+) found 738.0756, observed 738.0763.

2-*O*-sulfonato-β-D-glucopyranosyluronate-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside (1): Pd/$(OH)_2$ on carbon (Degussa type, 20%, 1.5 times the weight of starting material) was added to the solution of compound **S5** (13.8 mg, 0.017 mmol) in *t*-BuOH and H_2O (1/1, v/v, 2 mL) and then placed under an atmosphere of hydrogen. The reaction stirred for 16 h until C18 TLC (H_2O/acetonitrile, 10/90, v/v) indicated completion of the reaction. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo.* The residue was re-dissolved in water and then passed through a AG50W resin (Bio-Rad, 0.6 x 2.5 cm) using H_2O as eluent. Appropriate fractions were lyophilized to provide compound **1** in quantitative yield. ^{1}H NMR (600 MHz, D_2O) δ 5.08 – 5.04 (m, 1H, H1), 4.58 – 4.51 (m, 3H, H1', H2'), 3.95 (ddd, *J* = 10.7, 8.6, 2.6 Hz, 2H, H5', H2), 3.87 – 3.40 (m, 7H, H3, H4, H5, H6, H3', H4'), 1.90 (s, 3H, COCH_3. HRMS ESI-TOF: (M-Na^+) found 498.0535, observed 498.0541.

2-*O*-sulfonato-β-D-glucopyranosyluronate-(1→4)-2-*N*-sulfonato-2-deoxy-D-glucopyranoside (2): Pd/$(OH)_2$ on carbon (Degussa type, 20%, 1.5 times the weight of starting material) was added to the solution of compound **S6** (9 mg, 0.010 mmol) in *t*-BuOH and H_2O (1/1, v/v, 2 mL) and then placed under an atmosphere of hydrogen. The reaction was completed after 16 h as indicated by C18 TLC (H_2O/acetonitrile, 10/90, v/v). The mixture was filtered through Celite and the filtrate was concentrated *in vacuo.* The residue was re-dissolved in water and then passed through a short column of AG50W resin (Bio-Rad, 0.6 x 2.5 cm) using H_2O as eluent. Appropriate fractions were lyophilized to provide compound **2** quantitative yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.27 (d, *J* = 3.5 Hz, 1H, H1), 4.80 (t, *J* = 8.3 Hz, 1H, H2'), 4.52 (d, *J* = 8.1 Hz, 1H, H1'), 4.30 (t, *J* = 7.9 Hz, 1H, H4), 4.21 – 3.90 (m, 8H, H3, H4, H5, H6, H3', H4', H5'), 3.67 (dd, *J* = 5.6, 2.2 Hz, H2). HRMS ESI-TOF: (M-Na^+) found 557.9817, observed 557.9826.

Synthesis of Compounds 3 and 4

Dimethylthexylsilyl (methyl 2-*O*-levulinoyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (7): A suspension of donor **5** (900 mg, 1.86 mmol), acceptor **6** (860 mg, 1.55 mmol) and activated molecular sieves (4Å crushed, 1.5 g) in dichloromethane (19 mL) was stirred at ambient temperature under an atmosphere of argon for 1 h. The mixture was cooled to 0 ^{o}C followed by the addition of NIS (833 mg, 3.72 mmol) TfOH (17 *μ*l, 0.19 mmol). The reaction mixture was allowed to warm to 5 ^{o}C and after 15 min TLC analysis showed complete consumption of the glycosyl donor. The reaction mixture was neutralized with pyridine (~30 *μ*l), diluted with DCM and washed with aqueous $Na_2S_2O_3$ (saturated). The organic layer was dried (Mg_2SO_4), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane /EtOAc 4/1 v/v) to afford **7** (1.49 g, 83%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.46 (m, 1H, CH Aromatic), 7.38 – 7.24 (m, 7H, CH Aromatic), 6.62 (d, *J* = 6.7 Hz, 1H, NH), 5.47 (s, 1H, C*H* benzylidene), 5.27 (d, *J* = 7.9 Hz, 1H, H1'), 5.22 (d, *J* = 1.8 Hz, 1H, H5), 5.15 (d, *J* = 2.6 Hz, 1H,), 5.12 (s, 1H, H4'), 4.91 (dd, *J* = 2.5, 1.2 Hz, 1H, H2), 4.79 – 4.68 (m, 2H, CH_2Bn), 4.60 – 4.53 (m, 2H, H4, H3), 4.27 – 4.19 (m, 1H, H6a), 4.09 (dd, *J* = 8.7, 3.8 Hz, 1H, , H6b), 3.79 (d, *J* = 2.3 Hz, 1H'), 3.73 (dd, *J* = 4.3, 2.0 Hz, 1H, H3), 3.68 – 3.55 (m, 1H H2'), 3.49 (s, 1H, H5'), 3.37 (s, 3H, , COCH_3), 2.80 (m, *J* = 18.0, 8.0, 5.8 Hz, 2H, CH_2 Lev), 2.68 – 2.37 (m, 2H, CH_2 Lev), 2.21 – 2.12 (m, 3H CH_3 Lev), 2.04 (d, *J* = 1.6 Hz, 3H, CH_3 Ac), 1.63 (dt, *J* = 13.7, 7.0 Hz, 1H, C*H*$(CH_3)_2$), 0.97 – 0.75 (m, 12H, C(C$H_3)_2$ and CH(C$H_3)_2$), 0.26 – -0.05 (m, 6H, Si(C$H_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.06, 171.50, 170.02, 169.06, 161.71, 138.30, 137.92, 129.05,

128.35, 128.33, 127.71, 126.91, 126.06, 100.65, 93.77, 77.58, 77.36, 77.16, 76.83, 76.74, 74.82, 71.59, 71.08, 70.67, 69.64, 67.87, 66.77, 66.38, 66.14, 57.24, 52.11, 37.85, 34.18, 29.86, 27.84, 24.90, 20.87, 20.36, 20.19, 18.80, 18.73, 3.08. HRMS MALDI-TOF: ($M+Na^+$) found 996.2548, observed 996.2540.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (8): Hydrazine acetate (221 mg, 2.40 mmol) was added to a solution of compound **7** (471 mg, 0.48 mmol) in a mixture of ethanol and toluene (2/1, v/v, 6 mL). The reaction mixture was stirred at ambient temperature for 2 h, after which TLC analysis showed complete consumption of the starting material. The reaction mixture was diluted with dichloromethane, washed with water and brine, dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene/EtOAc 4/1 v/v) to afford **8** as an oil (434mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 – 6.98 (m, 10H, CH Aromatic), 5.47 (d, *J* = 5.1 Hz, 1H, C*H* benzylidene), 5.32 – 5.24 (m, 2H, H1', H4), 5.22 (t, *J* = 2.9 Hz, 1H, H5), 5.16 (d, *J* = 6.7 Hz, 1H, H1), 4.69 – 4.61 (m, 4H, CH_2Ph, H3', H4'), 4.60 – 4.55 (m, 1H, 3H), 4.28 – 4.04 (m, 2H, H6a ,H6b), 3.81 – 3.58 (m, 3H, H3, H2, H2'), 3.55 – 3.46 (s, 1H, H5'), 3.36 (d, *J* = 4.8 Hz, 3H, CH_3 COOMe), 2.66 – 2.57 (m, 5H), 2.35 (s, 3H), 2.05 – 1.98 (m, 3H, CH_3 Ac), 1.83 (s, 1H), 1.69 – 1.55 (m, 1H, CH(CH_3)$_2$), 1.26 (s, 1H), 0.91 – 0.79 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.21 – 0.10 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.88, 169.23, 169.20, 161.68, 137.98, 137.94, 137.90, 129.11, 128.96, 128.42, 128.35, 128.31, 128.24, 127.84, 127.08, 126.05, 125.38, 103.44, 100.55, 93.80, 92.48, 77.58, 77.16, 77.04, 76.74, 74.80, 74.11, 71.45, 69.51, 69.04, 67.23, 66.59, 66.36, 57.04, 52.10, 34.09, 29.60, 25.10, 24.84, 21.54, 20.80, 20.27, 20.14, 18.72, 18.67, -1.67. HRMS MALDI-TOF: ($M+Na^+$) found 898.2179, observed 898.2185.

Dimethylthexylsilyl *O*-(methyl-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (9): A suspension of compound **8** (220 mg, 0.247 mmol) and activated molecular sieves (4Å, 220 mg) in dichloromethane (3.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled (-78 ^{o}C) followed by addition of Et_3SiH (118 µl, 0.740 mmol) and TfOH (74 µl, 0.840 mmol). After stirring for 1 h at -78 ^{o}C, Et_3N (1 mL) and MeOH (1 mL) were added successively, and the mixture was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ (10%), dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/ MeOH 95/5 v/v) to afford compound **9** as an oil (135mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 10H, C*H* Aromatic), 6.93 (d, *J* = 7.8 Hz, 1H, NH), 5.29 (s, 1H), 5.27 – 5.20 (m, 2H, H1', H5'), 5.02 (dd, *J* = 10.7, 5.8 Hz, 2H, H1, H4'), 4.76 – 4.63 (m, 2H, CH_2 Bn), 4.58 – 4.54 (s, 2H, CH_2 Bn), 4.28 (dd, *J* = 10.8, 3.3 Hz, 1H, H3), 4.14 (t, *J* = 3.6 Hz, 1H, H4), 3.82 – 3.65 (m, 9H, H2', H3', H2, H5, H6a, , H6b, CH_3 COOMe), 2.06 – 1.98 (m, 3H, CH_3 Ac), 1.70 – 1.53 (m, 1H, CH(CH_3)$_2$), 0.92 – 0.78 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.23 – 0.10 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.60, 165.21, 141.29, 141.04, 131.81, 131.73, 131.26, 131.01, 130.98, 130.92, 105.44, 97.91, 95.88, 81.42, 80.80, 80.58, 80.38, 79.95, 78.61, 76.97, 76.45, 76.16, 72.99, 72.69, 72.23, 71.81, 71.62, 60.36, 55.78, 37.25, 28.07, 24.06, 23.48, 23.30, 21.96, 21.88, 1.63. HRMS MALDI-TOF: ($M+Na^+$) found 900.2335, observed 900.2331.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (10): A suspension of compound **8** (214 mg, 0.243 mmol) and activated molecular sieves (4Å, 220 mg) in dichloromethane (3.0 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled (-78 ^{o}C) followed by the addition of Et_3SiH (118 µl, 0.740 mmol) and $PhBCl_2$ (110 µl, 0.840 mmol). After stirring for 1 h at -78 ^{o}C, Et_3N (1 mL) and MeOH (1 mL) were added successively, and the mixture was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ (satd), dried ($MgSO_4$), filtered and the filtrate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography

(DCM/MeOH 95/5 v/v) to afford compound **10** as an oil (173 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.11 (m, 10H, CH Aromatic), 6.95 (d, *J* = 8.1 Hz), 5.23 – 5.04 (m, 2H, H1', H5'), 4.82 (ddd, *J* = 32.1, 16.4, 5.2 Hz, 3H, H1, H4', CH*H*Bn), 4.57 (ddd, *J* = 17.8, 13.4, 10.1 Hz, 3H, CH*HBn*), 4.31 – 3.83 (m, 3H, H3, H2), 3.78 (d, *J* = 2.8 Hz, 1H, H4), 3.69 – 3.54 (m, 4H, H3', H2', H5, H6a), 3.42 (d, *J* = 4.2 Hz, 1H, H6b), 2.03 – 1.87 (m, 3H CH_3 Ac), 1.62 – 1.42 (m, 1H, C*H*$(CH_3)_2$), 0.84 – 0.67 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.08 – 0.01 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.82, 171.40, 169.55, 169.44, 162.19, 138.39, 137.60, 133.88, 133.76, 128.69, 128.58, 128.50, 128.37, 128.24, 128.08, 128.04, 127.97, 127.89, 127.85, 127.69, 102.76, 95.15, 92.70, 79.30, 77.58, 77.16, 76.74, 75.79, 75.52, 75.16, 74.91, 74.54, 74.05, 73.35, 69.82, 69.59, 68.78, 61.88, 60.56, 57.63, 52.59, 52.55, 33.94, 29.65, 24.81, 21.17, 20.79, 20.23, 20.11, 18.69, 18.66, 14.30, -1.42. HRMS MALDI-TOF: (M+Na^+) found 900.2333, observed 900.2338.

Dimethylthexylsilyl *O*-(methyl-3-O-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (11): A suspension of Zn-Cu couple (2 g) was added to a solution of disaccharide **9** (138 mg, 0.157 mmol) in acetic acid (3.0 mL) under an atmosphere of Ar and the resulting mixture was stirred for 5 h. The mixture was then filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/ MeOH 95/5 v/v) to afford compound **11** as an oil (88 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.22 (m, 10H, CH Aromatic), 5.84 (d, *J* = 7.8 Hz, 1H, N*H*), 5.27 – 5.14 (m, 2H, H1', H5'), 5.01 – 4.84 (m, 2H, H1, H5), 4.82 – 4.64 (m, 2H, CH_2Bn), 4.59 – 4.51 (s, 2H, CH_2Bn), 4.28 – 4.01 (m, 2H, H3, H4), 3.83 – 3.56 (m, 9H, H2', H3', H2^A, H5, H6a, , H6b, CH_3 COOMe), 2.10 – 1.87 (m, 3H, CH_3 Ac), 1.61 (m, *J* = 7.0 Hz, 1H, C*H*$(CH_3)_2$), 0.94 – 0.75 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.23 – 0.09 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.11, 171.38, 169.60, 169.58, 138.25, 138.14, 128.59, 128.51, 128.47, 127.89, 127.82, 127.77, 127.70, 102.39, 95.37, 78.96, 77.58, 77.16, 76.85, 76.74, 73.66, 73.57, 73.38, 70.75, 70.15, 69.84, 69.41, 68.78, 60.54, 55.82, 53.56, 52.42, 34.19, 24.92, 23.73, 21.18, 20.89, 20.81, 20.22, 20.18, 18.69, 18.67, 14.32, 1.56. HRMS MALDI-TOF: (M+Na^+) found 798.3502, observed 798.3511.

Dimethylthexylsilyl *O*-(methyl-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (12): A suspension of Zn-Cu couple (2 g) was added to a solution of disaccharide **10** (140 mg, 0.157 mmol) in acetic acid (3.0 mL) under an atmosphere of Ar. The resulting reaction mixture was stirred for 5 h after which it was filtered through a pad of celite and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH 95/5 v/v) to afford compound **12** as an oil (100 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.22 (m, 10H, C*H* Aromatic), 6.11 (d, *J* = 7.9 Hz, 1H, N*H*), 5.24 (d, *J* = 5.3 Hz, 1H, H1'), 5.17 (t, *J* = 4.7 z, 1H, H4'), 4.94 (d, *J* = 7.7 Hz, 1H, H1), 4.90 – 4.55 (m, 5H, H5', C*H*HBn CH*H*Bn), 4.12 (q, *J* = 7.2 Hz, 1H, H3), 3.97 – 3.63 (m, 5H, H2', H3', H4, H5, H6a), 3.59 – 3.43 (m, 1H, H6b), 2.32 – 1.84 (m, 6H, CH_3 Ac, CH_3 AcN), 1.70 – 1.49 (m, 1H, C*H*$(CH_3)_2$), 0.96 – 0.71 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.25 – 0.10 (m, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.84, 179.22, 176.61, 173.76, 170.54, 170.00, 169.97, 169.90, 169.81, 169.51, 158.67, 149.17, 138.50, 138.31, 138.23, 138.05, 137.82, 129.40, 128.62, 128.58, 128.56, 128.53, 128.29, 128.17, 128.08, 128.02, 127.98, 127.94, 117.17, 103.53, 102.83, 96.30, 95.32, 90.38, 81.72, 79.87, 77.58, 77.37, 77.16, 77.02, 76.86, 76.74, 74.95, 74.89, 74.67, 74.29, 74.18, 73.84, 73.74, 73.19, 70.98, 70.35, 70.31, 70.19, 69.78, 69.54, 61.94, 61.83, 60.10, 59.41, 56.79, 52.82, 52.66, 37.60, 34.16, 34.09, 34.04, 32.07, 29.85, 24.96, 24.93, 23.83, 22.84, 21.21, 21.00, 20.89, 20.85, 20.57, 20.26, 20.22, 20.14, 20.09, 19.53, 18.71, 18.68, 18.63, 18.57, 14.34, 14.27, 0.14, -1.42. HRMS MALDI-TOF: (M+Na^+) found 798.3500, observed 798.3509.

Dimethylthexylsilyl *O*-(methyl-2-*O*-sulfonato-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-4-*O*-sulfonato-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside disodium salt (13): Sulfur trioxide pyridine complex (110 mg, 0.690 mmol) was added to a solution of the compound **11** (0.058 mmol, 45 mg) in DMF (1.5 mL)

and the resulting mixture was stirred for 2 h at ambient temperature. TLC analysis ($CHCl_3$/CH_3OH 9/1 v/v) indicated complete consumption of the starting material. Pyridine (0.2 mL) and methanol (0.5 mL) were added to the reaction mixture and stirred for an additional 30 min. The mixture was concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was passed through a column of iatrobeads (1.5 g, CH_3OH/$CHCl_3$ 96/4 to 88/12 v/v, containing 0.2% pyridine). The fractions containing product were concentrated *in vacuo* (bath temperature 20 °C), and the residue was immediately passed through a column of AG50W resin (Bio-Rad, , 0.6 × 5 cm, CH_3OH), providing the compound **13** as an oil (46 mg, 81%). ^1H NMR (600 MHz, CD_3OD) δ 7.82 (d, *J* = 13.2 Hz, 1H, *CH* Aromatic), 7.73 (d, *J* = 2.6 Hz, 1H, *CH* Aromatic), 7.28 (t, *J* = 8.9 Hz, 2H, *CH* Aromatic), 7.16 (ddd, *J* = 15.3, 14.2, 7.5 Hz, 6H, *CH* Aromatic), 7.10 – 7.04 (m, 2H, *CH* Aromatic), 5.49 (d, *J* = 2.3 Hz, 1H, H5'), 5.05 (s, 1H, H1'), 4.96 (dd, *J* = 7.9, 5.3 Hz, 2H, H4'), 4.66 (dd, *J* = 20.0, 7.6 Hz, 2H, H1,CH*H*Bn), 4.54 – 4.48 (m, 1H, C*H*HBn), 4.44 – 4.34 (m, 2H, CH*H*Bn, C*H*HBn), 4.22 (d, *J* = 2.3 Hz, 1H, H2'), 3.82 (t, *J* = 2.9 Hz, 1H, H3'), 3.69 (ddd, *J* = 17.9, 10.6, 5.6 Hz, 2H, H6a, H6b), 3.61 (dd, *J* = 7.5, 4.0 Hz, 1H, H5), 3.55 (d, *J* = 10.3 Hz, 3H, C*H*$_3$ COOMe), 3.17 (d, *J* = 6.5 Hz, 1H), 1.88 – 1.78 (m, 7H, C*H*$_3$Ac, C*H*$_3$ AcN), 1.50 – 1.41 (m, 1H, CH$(CH_2)_3$), 0.76 – 0.65 (m, 13H, C$(CH_2)_3$ and CH$(CH_2)_3$), 0.04 – -0.04 (m, 7H, Si$(CH_2)_3$). ^{13}C NMR (125 MHz, CD_3OD) δ 155.34, 149.28, 127.95, 127.44, 127.14, 102.71, 76.09, 73.73, 72.82, 71.88, 71.44, 71.43, 71.42, 71.41, 70.84, 70.42, 70.40, 67.61, 66.38, 51.31, 51.03, 51.37, 33.94, 29.31, 22.10, 22.08, 19.35, 19.30, 19.25, 19.21, 18.86, 17.70, 9.91. HRMS ESI-TOF: (M-2Na^++2H^+) found 956.2490, observed 956.2487.

Dimethylthexylsilyl (methyl-2-*O*-sulfonato-3-*O*-benzyl-4-*O*-acetyl-α-L-idopyranosyluronate)-(1→3)-6-*O*-sulfonato-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside disodium salt (14): Sulfur trioxide pyridine complex (367 mg, 2.31 mmol) was added to a stirred solution of compound **12** (0.115 mmol, 90 mg) in DMF (4.0 mL) at ambient temperature for 2 h. TLC analysis ($CHCl_3$, CH_3OH 90/ 10, v/v) indicated complete consumption of starting material. Pyridine (0.2 mL) and methanol (0.5 mL) were added to the reaction mixture, and the solution was continued to stir for an additional 30 min. The mixture was concentrated *in vacuo* (bath temperature 20 ^oC), and the residue was passed through a column of iatrobeads (1.5 g, CH_3OH/$CHCl_3$ 96/4 to 88/12 v/v, containing 0.2% pyridine). The fractions containing product were concentrated *in vacuo* (bath temperature 20 °C), and the residue was immediately passed through a column of AG50W resin (Bio-Rad, 0.6 × 5 cm, CH_3OH) providing compound **14** as an oil (104 mg, 92%). ^1H NMR (500 MHz, CD_3OD) δ 7.4 – 7.17 (d, 2H, CH Aromatic), 7.12 – 6.97 (m, 8H, CH Aromatic), 5.15 (s, 1H, H1$^{'}$), 4.96 (t, *J* = 9.0 Hz, 1H, H5'), 4.86 (d, *J* = 1.9 Hz, 1H, H4'), 4.78 (m, 2H, H1, CH*H*Bn), 4.64 – 4.54 (d, 1H, C*H*HBn), 4.47 – 4.35 (m, 3H, C*H*HBn, CH*H*Bn, H2'), 4.03 – 3.87 (m, 4H, H3', H6a, H6b, H3), 3.79 – 3.75 (m, 1H, H4), 3.69 (t, *J* = 6.8 Hz, 1H, H5), 3.54 (s, 4H, C*H*$_3$ COOMe, H2), 1.96 – 1.81 (m, 7H, C*H*$_3$Ac, C*H*$_3$AcN), 1.47 (dt, *J* = 13.8, 7.0 Hz, 1H, CH$(CH_2)_3$), 0.79 – 0.65 (m, 12H, C$(CH_3)_2$ and CH$(CH_3)_2$), 0.09 – -0.07 (m, 7H, Si$(CH_3)_2$). ^{13}C NMR (125 MHz, CD_3OD) δ 127.82, 127.52, 67.98, 66.27, 72.07, 72.06, 74.35, 70.10, 72.35, 63.79, 63.81, 63.80, 75.75, 75.67, 72.87, 51.89, 53.94, 48.65, 48.15, 35.92, 30.61, 22.71, 19.66, 14.18, 14.20, 18.13, 18.09, 19.60. HRMS ESI-TOF: (M-2Na^++2H^+) found 956.2488, observed 956.2481.

2-*O*-sulfonato-3-*O*-benzyl-α-L-idopyranosyluronate-(1→3)-4-*O*-sulfonato-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (15): A premixed solution of aqueous H_2O_2 (30%, 251 µL, 10.20 mmol) and 1 M LiOH (2.25 mL, 2.25 mmol) were added to a solution of compound **13** (45 mg, 0.045 mmol) in THF (1.0 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to attain pH 14). The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (3.5 g, H_2O/CH_3OH 9/1 to 7/3 v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of AG50W resin (Bio-Rad, , 0.6 × 5 cm) using CH_3OH as the eluent to provide the required compound

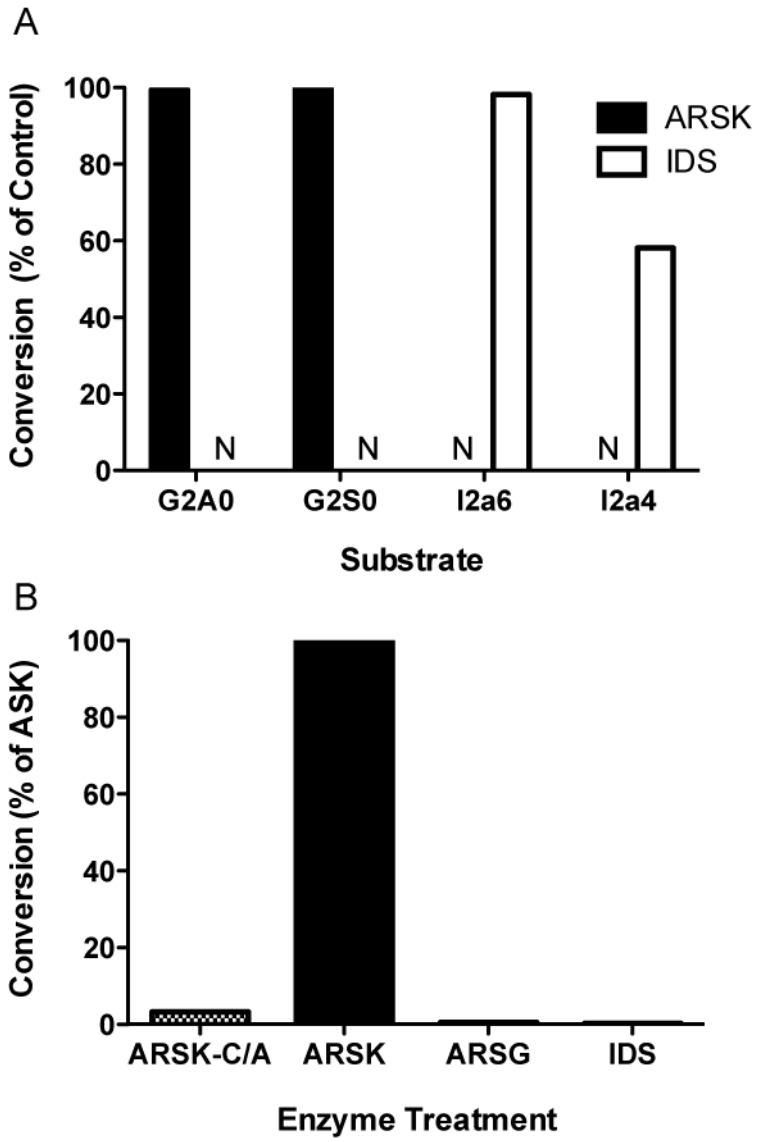
as as an oil (40 mg, 93%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.30 (d, *J* = 7.5 Hz, 2H, *CH* Aromatic), 7.20 – 7.04 (m, 7H, *CH* Aromatic), 5.11 (s, 1H, H1'), 4.74 (dd, *J* = 4.4, 2.2 Hz, 2H, H5', H4), 4.63 (t, *J* = 14.2 Hz, 2H, CH*H*Bn, H1), 4.53 (d, *J* = 12.4 Hz, 1H, C*H*HBn), 4.39 (q, *J* = 11.8 Hz, 2H, , CH*H*Bn, C*H*HBn), 4.23 (t, *J* = 2.2 Hz, 1H, H2'), 3.91 (t, *J* = 2.7 Hz, 1H, H4'), 3.87 – 3.78 (m, 2H, H2, H3), 3.75 – 3.62 (m, 4H, H3', H5, H6a, H6b), 3.14 (p, *J* = 1.5 Hz, 3H, C*H*$_3$ COOMe), 1.77 (s, 3H C*H*$_3$ Ac), 1.45 (hept, *J* = 6.9 Hz, 1H, $CH(CH_2)_3$), 0.75 – 0.66 (m, 11H, $C(CH_2)_3$ and $CH(CH_2)_3$). 0.18 (d, 6H, $Si(CH_2)_3$). ^{13}C NMR (150 MHz,) δ 179.49, 177.52, 153.29, 143.22, 142.37, 132.78, 132.76, 132.54, 132.52, 132.51, 132.50, 132.48, 132.10, 132.06, 132.04, 131.69, 105.18, 105.08, 100.87, 99.71, 82.83, 80.75, 80.67, 79.73, 79.58, 78.28, 78.21, 77.51, 76.59, 76.52, 76.35, 76.27, 76.25, 76.17, 75.20, 74.32, 74.17, 71.65, 71.52, 58.29, 52.42, 52.37, 52.32, 52.27, 52.24, 52.21, 52.18, 52.13, 52.04, 52.00, 51.95, 51.93, 51.92, 51.91, 51.89, 51.85, 51.82, 51.81, 51.80, 51.78, 51.76, 51.73, 51.73, 38.70, 38.67, 29.11, 26.83, 26.76, 23.98, 23.95, 23.91, 22.41, 22.36, 1.96, 1.92, 1.87, 1.83, 0.14, 0.09, 0.05. HRMS ESI-TOF: (M-3Na$^+$+1H$^+$) found 956.2490, observed 956.2485. The carboxylic acid (40 mg, 0.042 mmol) was dissolved in pyridine (825 µL), THF (412 µL) and H_2O (100 µL) and the mixture was cooled (0 °C) followed by addition of HF-pyridine (229 µL). The resulting reaction mixture slowly allowed to war to room temperature and left stirring overnight. The mixture was passed through a Sephadex LH-20 column (CH_2Cl_2/MeOH 1/1 v/v), and the product containing fractions concentrated *in vacuo.* The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9/1 v/v) to afford compound **15** as a white amorphous solid (27 mg, 81%). ^{1}H NMR (500 MHz, D_2O) δ 7.33 – 7.07 (m, 10H, CH Aromatic), 5.16 (d, *J* = 15.9 Hz, 2H, H1', H1α), 4.67 (d, *J* = 10.6 Hz, 4H, H4α, H4β, H1β, C*H*HBn), 4.64 – 4.57 (m, 1H, CH*H*Bn), 4.52 (dd, *J* = 20.4, 11.2 Hz, 2H, C*H*HBn, CH*H*Bn), 4.45 – 4.32 (m, 2H, H5α, H2α), 4.20 (dd, *J* = 7.7, 4.2 Hz, 1H, H5'), 4.10 (dd, *J* = 12.7, 9.6 Hz, 3H, H2', H3', H5'), 3.89 (dd, *J* = 21.0, 6.6 Hz, 2H, H5β, H3β), 3.82 (m, *J* = 18.9, 11.2, 5.3 Hz, 3H, H6a, H6b, H4'), 1.99 (t, *J* = 8.3 Hz, 3H C*H*$_3$ AcN).^{13}C NMR (125 MHz, D_2O) δ 130.32, 129.07, 128.93, 128.68, 128.66, 101.09, 101.09, 95.39, 91.66, 79.1, 76.38, 76.38, 76.37, 75.44, 75.25, 74.77, 74.76, 74.74, 73.95, 72.92, 72.41, 72.39, 72.38, 71.03, 69.46, 69.05, 68.56, 67.76, 67.74, 66.75, 53.25, 49.86, 49.05, 24.08, 22.46. HRMS ESI-TOF: (M-2Na$^+$+1H$^+$) found 757.9000, observed 757.9007.

2-*O*-sulfonato-3-*O*-benzyl-α-L-idopyranosyluronate-(1→3)-6-*O*-sulfonato-4-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (16): A premixed solution of aqueous H_2O_2 (30%, 579 µL, 10.20 mmol) and 1 M LiOH (122 mg, 5.10 mmol) were added to a solution of compound **14** (100 mg, 0.102 mmol) in THF (1.5 mL). The resulting mixture was stirred at ambient temperature for 8 h. An aqueous solution of NaOH (0.5 to 1.0 mL, 4N) was added to aciece pH 14. The reaction mixture was stirred for additional 18 h at ambient temperature. The mixture was then treated with AcOH (pH 8-8.5), and was concentrated *in vacuo* (bath temperature 20 °C). The residue was vortexed with water and applied to a RP-18 column (3.5 g), which was eluted with a stepwise gradient of H_2O and CH_3OH (9/1 to 7/3, v/v). The appropriate fractions were concentrated *in vacuo* (bath temperature 20 °C), and the residue was passed through a column of AG50W resin (Bio-Rad, 0.6 × 5 cm) using CH_3OH as eluent, providing the target compound as an oil (71 mg, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.21 – 7.17 (m, 2H, C*H* Aromatic), 7.09 – 6.96 (m, 7H C*H* Aromatic), 5.23 (s, 1H, H1'), 4.70 (d, *J* = 3.6 Hz, 3H, H5', CH*H*Bn, H1), 4.54 m, *J* = 11.8 Hz, 2H, C*H*HBn, C*H*HBn), 4.45 – 4.32 (m, 2H, H2', CH*H*Bn), 4.23 – 4.12 (m, 2H, H4', H2), 3.99 (d, *J* = 7.1 Hz, 2H, H3', H3), 3.97 – 3.91 (m, 1H, H6a), 3.84 – 3.80 (m, 2H, H4, H5), 3.76 (dd, *J* = 11.7, 4.8 Hz, 1H, H6b), 1.95 (s, 3H, C*H*$_3$ Ac), 1.53 – 1.41 (m, 1H, $CH(CH_3)_2$), 0.71 (q, *J* = 7.1 Hz, 12H, $C(CH_3)_2$ and $CH(CH_3)_2$), 0.01 (d, *J* = 11.5 Hz, 7H, $Si(CH_3)_2$). ^{13}C NMR (125 MHz, CD_3OD) δ 128.97, 128.84, 128.77, 128.71, 128.44, 128.2, 128.19, 128.18, 128.09, 128.07, 127.92, 127.85, 127.4, 127.39, 127.33, 127.3, 127.28, 126.89, 126.75, 126.14, 101.73, 96.76, 77.3, 76.55, 76.31, 75.8, 75.6, 75.55, 75.54, 74.88, 74.86, 74.81, 74.81, 74.78, 73.63, 72.88, 72.22, 72.19, 72.18, 72.13, 71.81, 71.51, 71.49, 71.45, 71.41, 70.74, 70.74, 70.21, 70.03, 69.43, 69.27, 68.65, 68.51, 67.88, 67.12, 66.22, 48.08, 34.8, 34.79, 34.11, 34.1, 34.09, 34.08, 34.06, 34.05, 23.55, 22.74, 20.39, 20.25, 19.44, 18.72, 18.71, 17.93, 17.91, 17.11. HRMS

ESI-TOF: (M-2Na^++2H^+) found 956.2488, observed 956.2481. The carboxylic acid (33mg, 0.035 mmol) was dissolved in pyridine (600 µL), THF (300 µL) and H_2O (100 µL). The reaction was cooled (0 °C) followed by addition of HF-pyridine (229 µL) and the resulting reaction mixture was slowly warmed to room temperature and stirring was continued overnight. The mixture was passed through a Sephadex LH-20 column (CH_2Cl_2/MeOH 1/1 v/v), and the product containing fractions were concentrated *in vacuo* and the residue purified by silica gel column chromatography (CH_2Cl_2/MeOH 9/1 v/v) to afford compound **16** as a white solid (21.0 mg, 75%). ^{1}H NMR (500 MHz, D_2O) δ 7.31 – 7.12 (m, 8H, C*H* Aromatic), 7.09 (td, *J* = 7.4, 6.1, 3.0 Hz, 3H, C*H* Aromatic), 5.15 (d, *J* = 15.9 Hz, 1H, H1'α/β), 5.05 (d, *J* = 3.6 Hz, 1H, H1α), 4.67 (m, 2H, H5'α/β), 4.64 – 4.45 (m, 4H, H1β, C*H*HBn, C*H*HBn, CH*H*Bn), 4.44 – 4.30 (m, 3H, H2α, H2'α/β, CH*H*Bn), 4.18 (dd, *J* = 7.9, 4.2 Hz, 1H, H5α/β), 4.14 – 4.04 (m, 3H, H2β, H3α/β, H4'α/β), 3.93 – 3.74 (m, 6H, H3'α/β, H4α/β, H6aα/β), 3.70 – 3.61 (m, 1H, H6bα/β), 1.98 (d, *J* = 3.9 Hz, 4H, C*H*$_3$ Ac) ^{13}C NMR (125 MHz, D_2O) δ 130.09, 128.84, 128.7, 128.45, 128.44, 127.31, 100.88, 100.88, 95.18, 91.45, 78.89, 76.17, 76.17, 76.17, 75.24, 75.04, 74.57, 74.55, 74.54, 74.53, 72.72, 72.2, 72.19, 72.18, 70.83, 70.68, 69.27, 68.85, 68.36, 67.56, 67.54, 67.5, 66.55, 64.93, 53.06, 49.67, 48.86, 22.29. HRMS ESI-TOF: (M-2Na^++2H^+) found 757.9000, observed 757.9004.

2-*O*-sulfonato-α-L-idopyranosyluronate-(1→3)-4-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (3): A suspension of Pd/C (24 mg) was added to a solution of the **13** (12 mg) in a mixture of CH_3OH (2.5 mL) and H_2O (0.75 mL). The mixture was placed under an atmosphere of hydrogen, and the progression of the reaction was monitored by TLC (silica gel, $CHCl_3$/CH_3OH/H_2O 60/40/10, v/v/v; EtOAc/pyridine/water/CH_3COOH, 3/5/3/1, v/v/v). The residue was passed through a short column of AG50W resin (Bio-Rad, 0.6 × 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze dried to provide the final product **3** (7.3 mg, 78%). ^{1}H NMR (800 MHz, D_2O) δ 5.25-5.18 (d, 2H, H1'α,β), 5.17 (d, J = 3.6 Hz, 1H, H1α), 4.86 (dd, J = 5.1, 1.8 Hz, 2H, H5'), 4.73 – 4.71 (m, 1H, H1β), 4.70 (d, J = 2.6 Hz, 1H, H4α), 4.66 (s, 1H, H4β), 4.35 (dd, J = 11.1, 3.6 Hz, 1H, H2α), 4.23 (dd, J = 8.2, 4.1 Hz, 1H, H5α), 4.18 – 4.11 (m, 3H, H3α, H2'α,β), 4.02 (d, J = 4.9 Hz, 4H, H4'α,β, H3β,H2β), 3.96 (s, 2H, H3'α,β), 3.82(dd, J = 8.0, 4.2 Hz, 1H, H5β), 3.78 – 3.64 (m, 4H, H6a,α,β, H6b,α,β), 2.04 (d, J = 1.0 Hz, 6H, C*H*$_3$ AcN). ^{13}C NMR (125 MHz, D_2O) δ 101.45, 101.39, 95.45, 91.56, 79.94, 79.74, 76.8, 74.1, 73.12, 69.06, 68.71, 68.66, 68.23, 67.8, 52.5, 49.06, 22.28. HRMS ESI-TOF: (M-3Na^++1H^+) found 577.9139, observed 577.9144.

2-*O*-sulfonato-α-L-idopyranosyluronate-(1→3)-6-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside trisodium salt (4): A suspension of Pd/C (8.0 mg) was added to a solution of **16** (4 mg) in a mixture CH_3OH (0.8 mL) and H_2O (0.25 mL). The mixture was placed under an atmosphere of hydrogen, and the progress of the reaction was monitored by TLC (silica gel, $CHCl_3$/CH_3OH/H_2O 60/40/10, v/v/v; EtOAc/pyridine/water/CH_3COOH, 3/5/3/1, v/v/v). The residue was passed through a short column of AG50W resin (Bio-Rad, 0.6 × 2.5 cm) using H_2O as the eluent, and the appropriate fractions were freeze dried to provide the final product **4** (4.8 mg, 81%). ^{1}H NMR (800 MHz, D_2O) δ 5.12 (d, *J* = 3.7Hz, 1H, H1α), 5.11 and 5.17 (each s, 2H, H1'α and H1'β), 4.62 (d, *J* = 8.5 Hz, 1H, H1β),, 4.50 (dd, *J* = 4.7, 2.1 Hz, 2H, H5'α and β), 4.27 – 4.23 (m, 1H, H5α), 4.22 (dd, *J* = 11.1, 3.7 Hz, 1H, H2α), 4.12 – 4.00 (m, 8H, H4 α and β, H6α and β, H2'α and β), 3.98 – 3.91 (m, 3H, H3'α and β, H3α), 3.90 (t, *J* = 2.2 Hz, 2H, H4'α and β)), 3.85 (dd, *J* = 8.1, 4.1, Hz, 1H, H5β), 3.75 (dd, *J* = 10.9, 3.1 Hz, 1H, H3β), 1.96 (d, *J* = 1.2 Hz, 6H, C*H*$_3$ AcN). ^{13}C NMR (125 MHz, D_2O) δ 100.53, 100.45, 94.99, 91.46, 77.09, 76.61, 76.09, 74.62, 73.9, 73.15, 72.96, 70.51, 68.77, 68.73, 67.88, 61.33, 61.32, 61.31, 61.26, 53.46, 49.88, 49.88, 22.75, 21.16 HRMS ESI-TOF: (M-2Na^++2H^+) found 577.9144, observed 577.9147.



Supporting Information Figure S1. Specificity of ARSK for 2-sulfoglucuronate containing substrates. (A) G2A0, G2S0, I2a4 and I2a6 were incubated with ARSK or IDS as indicated. In each reaction, 2 nmol of substrate was mixed with either 5 ng of ARSK or 50 ng of IDS as indicated, and incubated overnight. Samples were then [$^{12}C_6$]aniline-tagged and mixed with a fixed amount (10 pmol) of [$^{13}C_6$]aniline-labeled substrate as standard. The samples were analyzed by LC/MS and the amount of substrate degraded was determined as a measure of product formation. (B) G2S0 was incubated with a mutant form of ARSK containing a Cys80 to Ala80 modification (ARSK-C/A, 5 ng), with wildtype ARSK (5 ng), with ARSG (30 ng), or with IDS (50 ng). Samples were then [$^{12}C_6$]aniline-tagged and mixed with a fixed amount (10 pmol) of [$^{13}C_6$]aniline-labeled substrate as standard. The samples were analyzed by LC/MS and the amount of substrate degraded was determined as a measure of product formation. Reactions that did not yield any product are marked by N = None

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